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4D optical coherence tomography of aortic valve dynamics in a murine mouse model ex vivo

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ABSTRACT

The heart and its mechanical components, especially the heart valves and leaflets, are under enormous strain during lifetime. Like all highly stressed materials, also these biological components undergo fatigue and signs of wear, which impinge upon cardiac output and in the end on health and living comfort of affected patients. Thereby pathophysiological changes of the aortic valve leading to calcific aortic valve stenosis (AVS) as most frequent heart valve disease in humans are of particular interest. The knowledge about changes of the dynamic behavior during the course of this disease and the possibility of early stage diagnosis could lead to the development of new treatment strategies and drug-based options of prevention or therapy.
ApoE-/- mice as established model of AVS versus wildtype mice were introduced in an ex vivo artificially stimulated heart model. 4D optical coherence tomography (OCT) in combination with high-speed video microscopy were applied to characterize dynamic behavior of the murine aortic valve and to characterize dynamic properties during artificial stimulation.
OCT and high-speed video microscopy with high spatial and temporal resolution represent promising tools for the investigation of dynamic behavior and their changes in calcific aortic stenosis disease models in mice.
Keywords: optical coherence tomography, aortic valve, 4D imaging

1. INTRODUCTION

One of the most common heart valve diseases is the calcific aortic valve stenosis, which shares many risk factors with atherosclerosis like older age, male gender, smoking, obesity and hypertension [1]. AVS is a significant public health problem and a major cause of morbidity and mortality in North America and Europe in elderly adults [2] and becomes more important due to the prospective demographic development.

The aortic valve is composed of three semilunar cusps and maintains unidirectional blood flow from the left ventricle into the aorta during each cardiac cycle. The aortic valve operates in a highly strained hemodynamic environment and undergoes enormous strain during the more than three billion heart beats over the averages persons´s lifetime.

The three basic loading states affecting the aortic valve are flexure, shear and tension [3, 4]. All of them influence valve remodeling processes or pathological changes which decrease valve function and cardiac output. Until now, little is known about the pathogenesis and progression of AVS because the clinical manifestation of the disease is preceded by a mostly undetected multi-year process of inflammation and fibrotic changes leading to thickened and stiff valve cusps with decreased flexibility and left ventricular outflow obstruction. Aortic valve disease is characterized by fibrotic disorganisations of the extracellular matrix. Due to collagen production and elastin fragmentation, the elasticity of the valve tissue decreases and further progression leads to calcification with still preserved normal valve function. Compensatory mechanisms drive heart muscle hypertrophy and the disease becomes symptomatic [3]. At this point, untreated AVS becomes fatal in an average of 5 years. Hence, the surgical replacement of the aortic valve, with either mechanical or biological valve prostheses, is the only curative treatment affecting annually 275,000 patients worldwide [5].

To understand the mechanics of disease formation and progression, animal models provide powerful tools. Beside rabbit and swine, mouse is the most common species used [3] to study valve biomechanics as well as the progression of AVS. Although mechanics of murine valves are difficult to test due to their small size, mouse models offer the advantages to demonstrate the progression of AVS within short timeframes and feature significant benefit providing genetic knockouts. Under high fat/high cholesterol diet, ApoE-/- mice develop hyperlipidemia, thickened cusps, activated endothelial cells and subendothelial lesions and represent an excellent tool to investigate potential key molecular modulators of heart valve disease.

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Due to their small size, tissue structures of mouse models are difficult to investigate with non-invasiv imaging techniques. To visualize small changes in tissue geometry due to pathological effects, high-resolution imaging techniques are necessary. Therefore, we present a novel approach for the characterization of biomechanical properties of murine aortic heart valves by using optical coherence tomography (OCT) and high-speed video microscopy in an ex vivo murine heart model.

The objective of this study is to show the feasibility of these imaging techniques to visualize aortic valve dynamics during uninterrupted artificial stimulation.The advantage of the presented imaging techniques is the high temporal and spatial resolution in 2D for video microscopy and 3D for OCT by using a low-speed OCT system.

2. METHODOLOGY

2.1 Ex vivo heart preparation

All experiments were approved by the animal care and use committee of the local government authorities and were performed in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 7th edition, 1996). In this study, ten 17-week-old C57BL/6J wildtype and seven 12-month-old ApoE knockout mice on C57BL/6J background were used.

The mice were anesthetized and heparinized. Afterwards, they were sacrificed by cervical dislocation and the hearts were removed immediately and rinsed in PBS to remove the entire blood. The aorta was shortened as much as possible to assure an optimal view of the OCT scanner head onto the aortic valve cusps. Finally, the apex of the heart was cut off and a catheter was inserted into the left ventricular outflow tract to mimic the normal flow direction through the aortic valve with a custom-made pump. The heart was fixed onto a catheter by a nylon suture to prevent sliding off due to the induced pressure during artificial stimulation of the valve.

2.2 Experimental setup and artificial stimulation of aortic valves

We developed a measurement setup for the visualization of aortic valve dynamics. As we found no suitable established heart model, we used an experimental pump device [6] for artificial stimulation of the aortic valve and a gating technique for time resolved high-resolution three-dimensional imaging of tissue movement with optical coherence tomography and intravital microscopy [7]. The experimental setup is shown in Fig. 1. The main components are two independent syringe pumps, one to insert and one to withdraw fluid (PBS), and two pinch valves for controlling the flow direction. PBS (phosphate buffered saline solution) is used instead of blood to avoid scattering loss and image artifacts for the near-infrared light based OCT. Stimulation is pressure-controlled following a sinusoidal pattern with a frequency of 50 beats per minute, which is one magnitude lower than the physiological heart beat but in the absence of other appropriate stimulation setups, it is suitable to show differences between healthy and calcified tissue dynamics and the advantage of OCT imaging. Pressure limits were adjusted individually to each heart in such a way that the pumped volume of each cycle is approximately 40 µl to mimic the normal stroke volume through the valve. To mimic the closing of the valve due to the blood volume in the aorta, one of the two pumps withdraws fluid from the heart until the pressure matches the lower pressure limit.

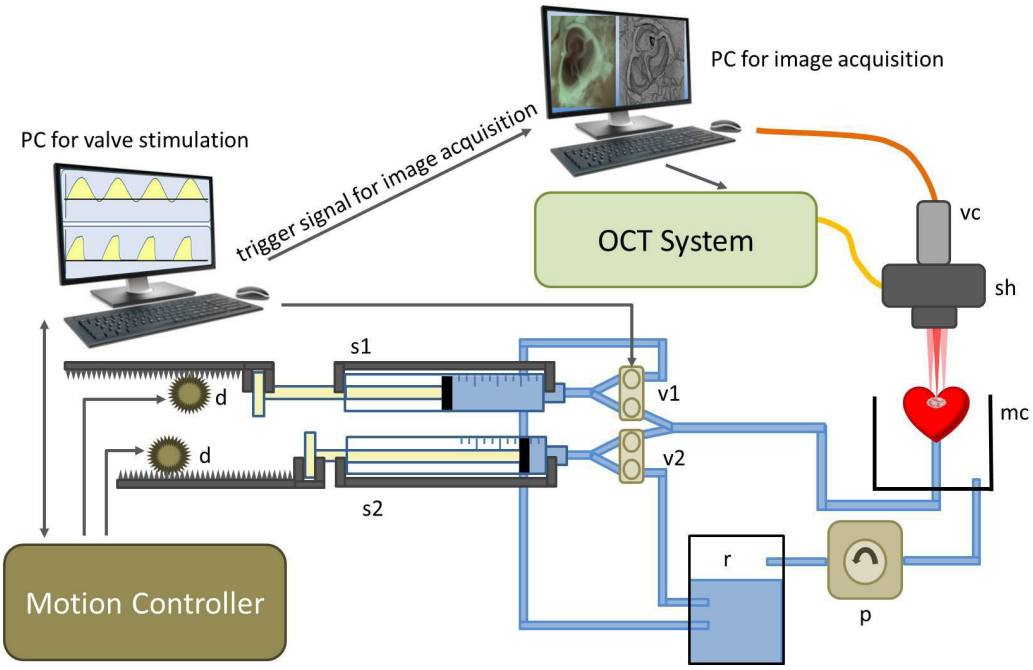

Fig. 1. Experimental setup for 4D visualization of aortic valves in mice. The setup consists of two independent pumps (s1 and s2) to perform the opening and closing of the aortic valve during artificial stimulation. The scanner head (sh) enables the simultaneous acquisition of the aortic valve with 4D OCT and white-light microscopy using a high-speed video camera. Other components: mc... measurement chamber; p… peristaltic pump; r… reservoir; d… linear drive, v1 and v2…magnetic valves

2.3 Imaging system and image acquisition

A spectral domain OCT system [8] with a center wavelength of 830 nm and a band width of 50 nm at half maximum was used with an A-scan rate of 12 kHz to visualize the structure and dynamics of the aortic valves *ex vivo*. For simultaneous visualization of aortic valve structures, OCT and video microscopy use the same beam path via a dichroic mirror. As the OCT system is not fast enough to acquire 3D images of the moving aortic valve, a scanning algorithm making use of the repetitive tissue movement due to the artificial stimulation was applied. Therefore, OCT cross-sections were continuously acquired without interruptions at the same position by deflecting just one galvanometer scanner. A trigger signal created from the pump device after each stimulation cycle sets the position of a second galvanometer scanner one step further and again cross-sections are continuously acquired at this new position.

After the measurement, the acquired OCT cross-sections were rearranged to form the different 3D image stacks showing the time resolved movement of the aortic valve cusps. High-speed video microscopy was performed with a Basler camera (acA2000-340kc) in which image sequence is acquired directly prior to the OCT measurement, because of the different measurement time and to avoid storage disturbance due to the high data transfer rates.

3. RESULTS

Fig. 2 shows an exemplary aortic valve with OCT and video microscopy under static conditions without artificial stimulation. Due to the three-dimensional image information, OCT provides a detailed visualization of all three cusps and the residual aortic boundary without any further sample preparation. It is also visible that OCT signal decreases rapidly within the aortic tissue, which is the reason why visualization through the intact aorta cannot be performed with this OCT system. Those data can be used for quantitative analysis of cusp thickness and valve opening area as it was performed during the dynamic measurements.

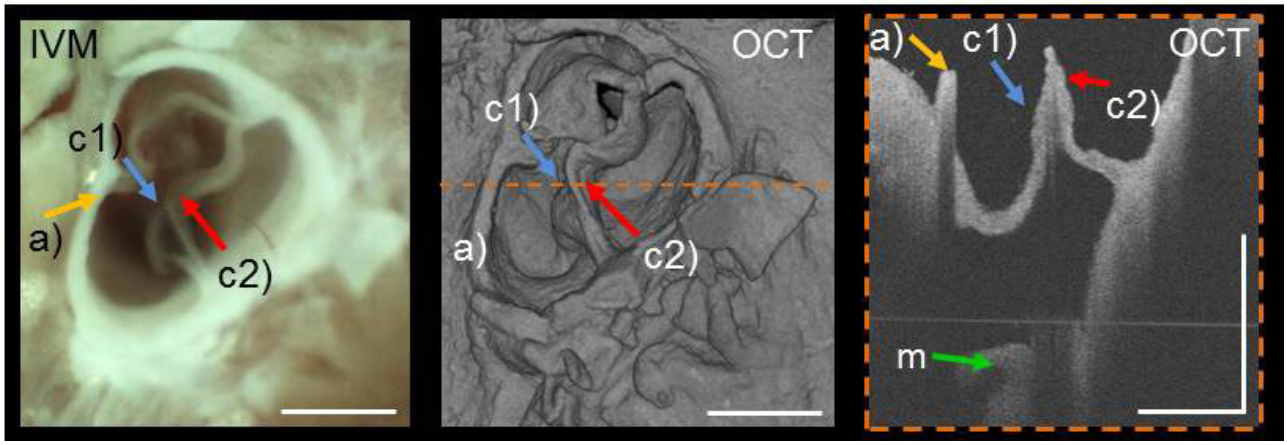

Fig. 2 ex vivo aortic valve structure imaged with IVM and OCT. The small structures of the three cusps are clearly visible. The advantage of OCT imaging is the three-dimensional information content, which allows different views like cross-sections without any sample preparation. This enables the easy analysis of structural differences in health and diseased tissue like cusp thickness. Scale bar corresponds to 500 µm. c1, c2… cusp one and two; a… residual aortic tissue; m… lower mitral leaflet

Exemplary findings of the dynamic measurements are shown in Fig. 3 where the aortic valve of a 17-week-old C57BL/6J and a 12-month-old ApoE knockout mouse was visualized under same conditions of artificial stimulation.
While all three cusps of the healthy young mouse open widely, opening movement of one cusp of the ApoE knockout mouse is terminated due to advanced calcification and AVS phenotype (see c1 of the ApoE knockout mouse in Fig. 3). Due to this late stage of the disease in the 12-month-old knockout mouse, the differences in the aortic structure are clearly visible between both mice regarding the thickness of the aortic tissue and the cusps and the maximum opening area of the valve (diagram on the right side of Fig. 3). While these differences are difficult to see in the video microscopy images, OCT provides a clear insight into the tissue structure and therefore enables the detailed study of pathological changes in morphology influencing tissue dynamics during stimulation.

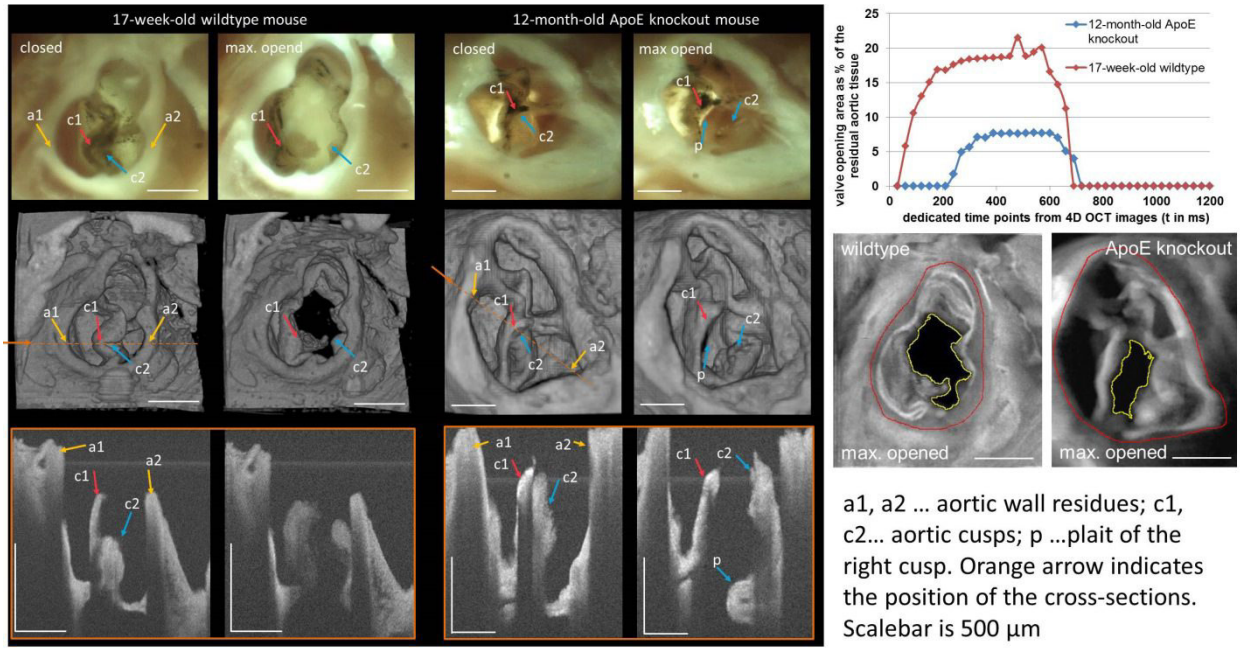

Fig. 3 Example images of the valve dynamics of a 17-week-old wildtype mouse and a 12-month-old female ApoE knockout mouse visualized with 4D OCT and high-speed video microscopy. It is clearly visible that the aorta tissue of the ApoE knockout mouse is thickened compared to the wildtype and the motility of the cusps is decreased. Furthermore, the measurement of the valve opening area (right diagram) shows significant differences due to the progression of calcification and aortic valve stenosis.

OCT enables the measurement and comparison of clinically relevant parameters like maximum opening area due to the 3D image information. This can be obtained in the third column of Fig. 2. While video microscopy and OCT enface images show little opening of the valve (compare last column), one can see in the cross-sectional view (third row) that the opening is much wider than it seems. Due to the movement, the plait (marked with p in Fig. 3) of the right cusp turns upwards and appears in the flow channel, which leads to the impression of a closed valve in the enface views of the ApoE knockout mouse. Therefore, the 3D image information from OCT is a useful tool for further detailed analysis of the dynamic behavior of aortic valve tissue. Thickness of the valvular tissue and information about dynamic properties like maximum opening area of the cusps and the slope of the opening and closing can be measured from these images. To show this advantage in an exemplary measurement comparing the young healthy and old calcified tissue, we segmented the residual aortic tissue and measured the opening area as percentage of the residual aorta, respectively. The results are shown in the diagram of Fig. 3. It becomes clearly visible that the healthy young wildtype valve opens wider and earlier compared to the diseased ApoE knockout valve. This seems to be in good agreement with the idea that stiffer and calcified tissue needs more pressure to open than the non-diseased motile valve tissue.

DISCUSSION

The presented results show that optical coherence tomography and high-speed video microscopy are promising tools for the investigation of dynamic behavior and its changes in calcific aortic valve stenosis disease models in mice. OCT offers an easy access to the tissue morphology in 3D and the measurement of tissue parameters like thickness without any sample preparation like staining or cutting.

OCT can be a helpful tool to observe drug therapies and novel approaches for the treatment of AVS in animal models. The high spatial and temporal resolution enables new insights into the course of this disease and may lead to a characterization and identification of early stages before significant hemodynamic obstruction has occurred and intervention by novel drug therapies is possible. This first feasibility study reveals further steps to enhance the experimental setup and imaging techniques to increase the information content of the measurements and thereby rising comparability of the results to clinically relevant parameters

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